

Developmental Cell, Volume 60

Supplemental information

Pancreatic alpha and beta cell fate choice is directed by apical-basal polarity dynamics

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Supplemental Figures

Figure S1, Related to Figure 1.

Figure S2. Related to Figure 1.

Figure S3. Related to Figures 2 and 3.

Figure S4. Related to Figure 4.

Figure S5. Related to Figure 5.

Figure S6. Related to Figures 5 and 6.

Supplemental Tables

Table S1, Related to STAR Methods

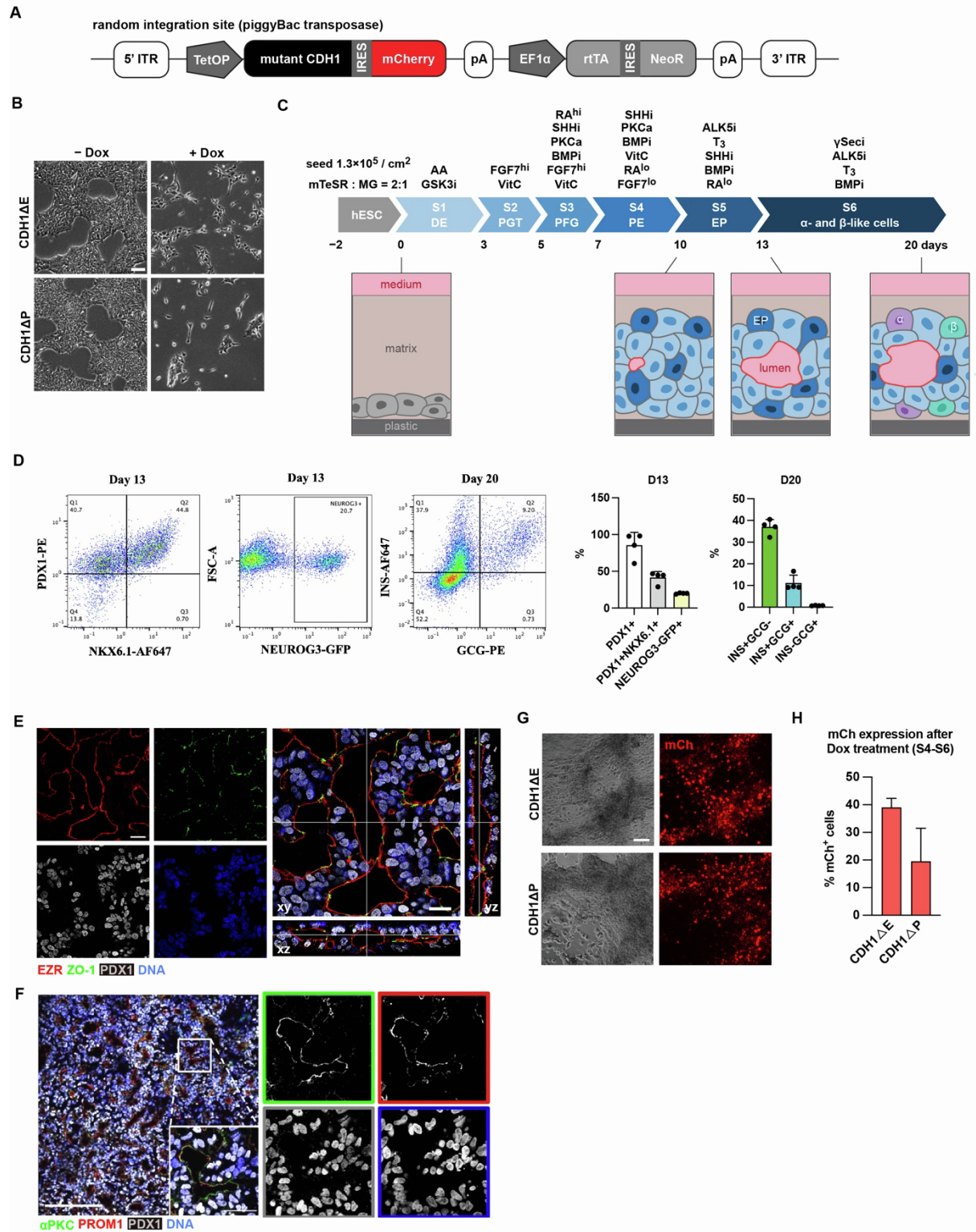
Table S2, Related to Figure 5

Table S3, Related to Figure 5

Supplemental Video

Video S1, Related to Figure 2.

Figure S1



Supplementary Figure S1. Dominant-negative CDH1 mutant cell line design and characterization and the Matrigel overlay differentiation protocol setup, related to Figure 1.

(A) Transgenic cassette inserted into hESCs for inducible and detectable overexpression of dominative-

negative CDH1 mutants. ITR, inverted terminal repeats. TetOP, Dox-responsive promoter. IRES, internal ribosomal entry site. pA, polyadenylation signal. EF1 α , constitutive promoter. rtTA, reverse tetracycline trans-activator. NeoR, neomycin resistance.

(B) Morphology changes upon mutant CDH1 overexpression. Phase contrast images of clonal hESC lines cultured under pluripotency-maintaining conditions. Dox, doxycycline (48h treatment). Scale bar, 100 μ m.

(C) Protocol for differentiation of hESCs into alpha and beta cells inside a 3D epithelial environment. DE, definitive endoderm. PGT, primitive gut tube. PFG, posterior foregut. PE, pancreatic endoderm. EP, endocrine progenitors. MG, Matrigel. i, inhibitor. a, activator. AA, activin A. VitC, vitamin C. RA, retinoic acid. SHH, Sonic Hedgehog. T3, triiodothyronine. γ Sec, gamma secretase.

(D) Representative flow cytometry density plots and quantification of PDX1, NKX6.1, NEUROG3, INS and GCG expression at Day 13 and Day 20 differentiating Matrigel overlay cultures. Data represent the mean \pm SEM (n = 4 independent repeats).

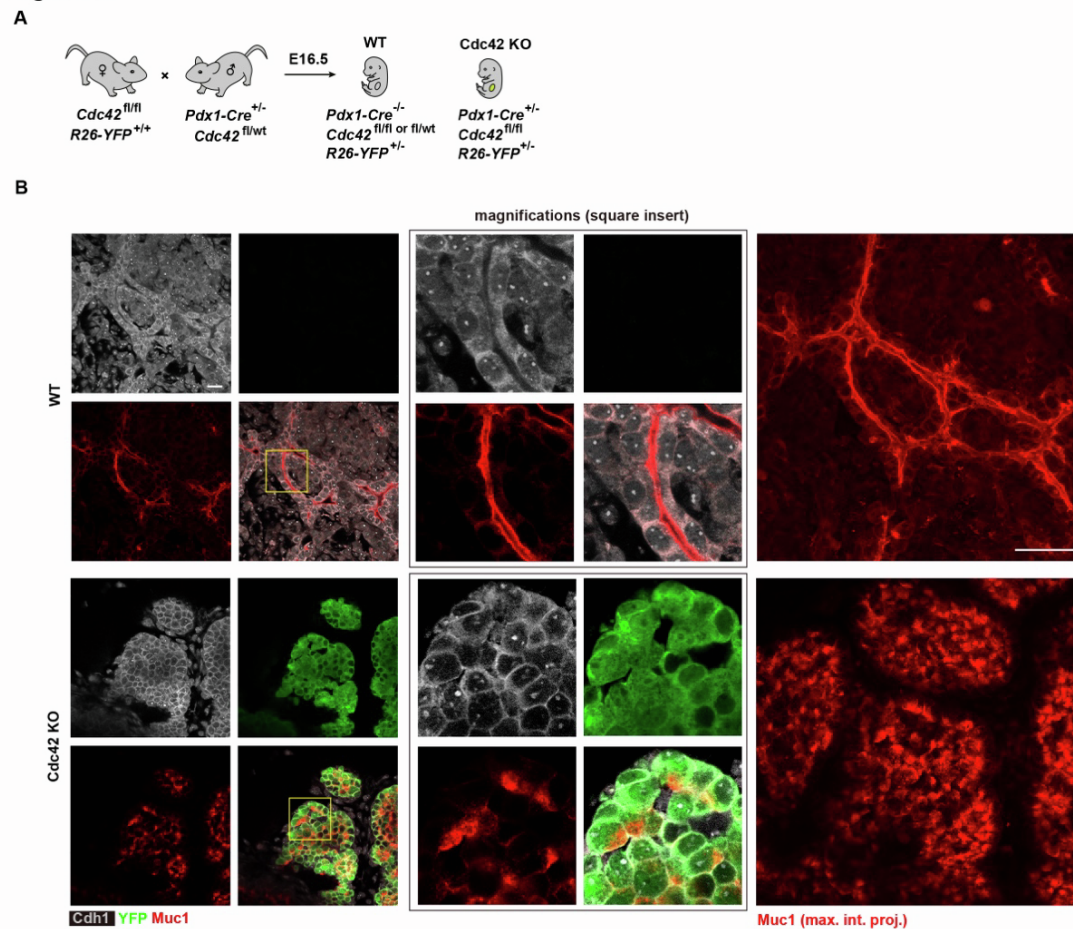
(E) Confocal image with orthogonal views of z-stack in S5 (Day 13) differentiating Matrigel overlay cultures. EZR, red. ZO-1, green. PDX1, gray. DNA (DAPI), blue. Scale bar, 25 μ m.

(F) Confocal image of polarity markers (α PKC and PROM1) in S5 (Day 13) differentiating Matrigel overlay cultures. PROM1, red. α PKC, green. PDX1, gray. DNA (DAPI), blue. Scale bar, 250 μ m.

(G) Mosaic expression of the mCherry reporter in CDH1 mutant cultures at S6 (treated with Dox for 13 days). Scale bar, 75 μ m.

(H) Flow cytometry quantification of transgenic (mCherry⁺) S6 cells (treated with Dox for 13 days). Data represent the mean \pm SEM (n = 5 independent repeats).

Figure S2

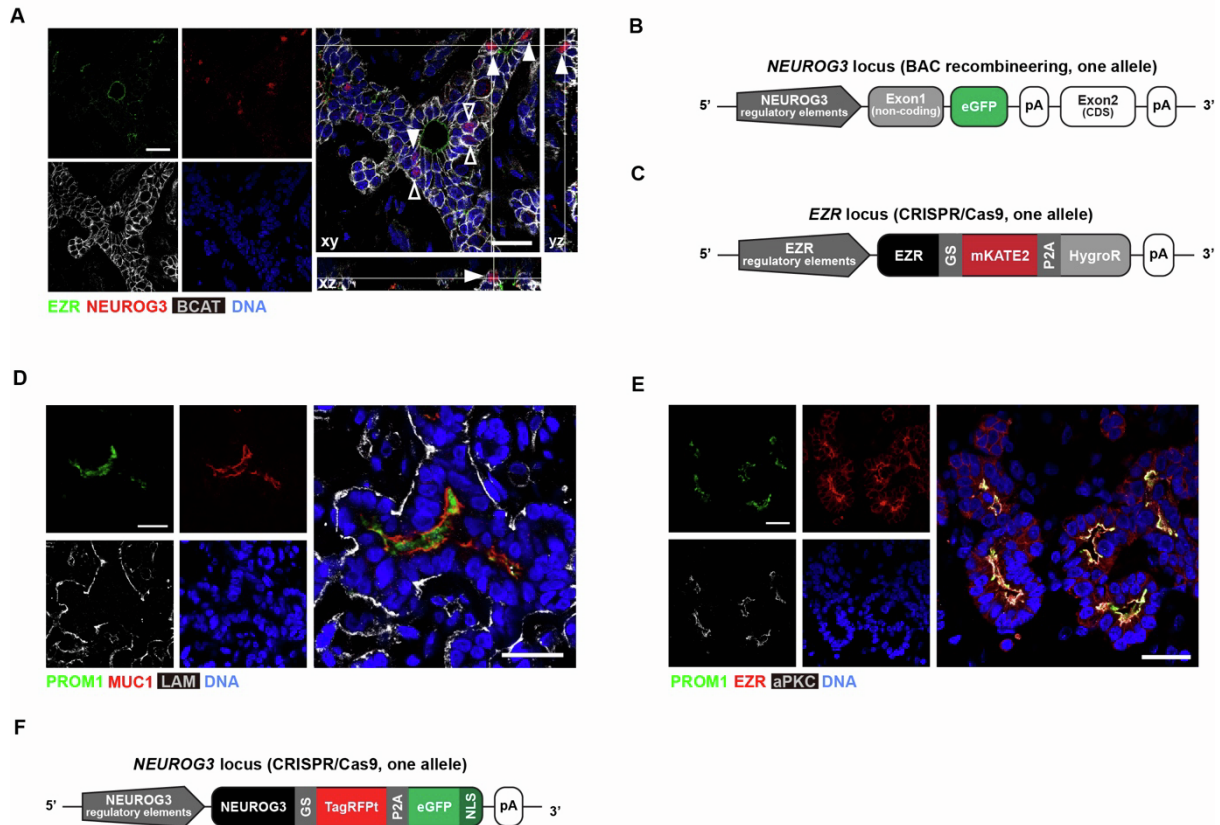


Supplementary Figure S2. *Cdc42* knockout disrupts apical-basal polarity in the developing mouse pancreas, related to Figure 1.

(A) Breeding setup for the generation of pancreatic (*Pdx1*-Cre driven) *Cdc42* knockout mice. fl, LoxP-flanked allele; R26, ROSA26 locus; YFP, yellow fluorescent protein; wt, wild-type control; KO, knockout.

(B) E16.5 wild-type and *Cdc42* knockout dorsal pancreas tissue. Confocal images and maximum intensity projection (max. int. proj.) of a z-stack. Cdh1, gray. Muc1 (Mucin1), red. R26-YFP, green. Scale bar, 250 μ m.

Figure S3



Supplementary Figure S3. Endocrine progenitor niche in the developing human pancreas, PROM1 is a surface marker of apical-basal polarity, and NEUROG3-EGFP/EZR-mKATE2 double reporter and NEUROG3-TagRFPtEGFP reporter cell line design, related to Figures 2 and 3.

(A) EP niche in the developing human pancreas. Confocal images of fetal pancreas sections (9.9 wpc) with orthogonal views of z-stack. Filled arrowheads, apical-basally polarized NEUROG3⁺ cells containing an EZR⁺ membrane. Empty arrowheads, non-polarized NEUROG3⁺ cells. EZR, green. NEUROG3, red. BCAT, gray. DNA (DAPI), blue. Scale bar, 25 μ m.

(B) Transgenic locus of the NEUROG3-EGFP reporter. BAC, bacterial artificial chromosome. EGFP, enhanced green fluorescent protein. pA, polyadenylation signal. CDS, coding sequence.

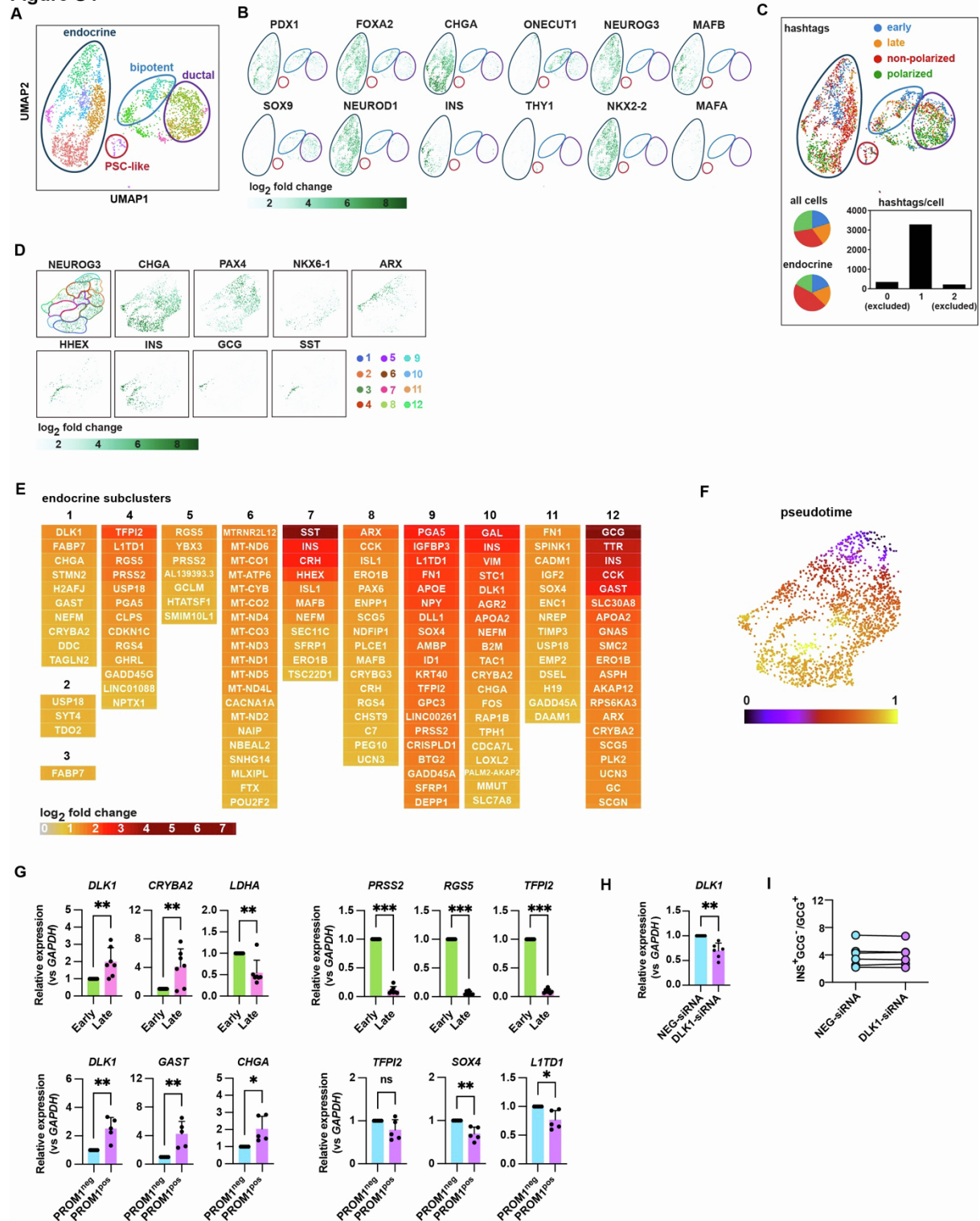
(C) Transgenic locus of the EZR-mKATE2 fusion reporter. GS, glycine-serine linker. mKATE2, red/far-red fluorescent protein. P2A, self-cleaving peptide. HygroR, hygromycin resistance.

(D and E) Apical membrane-specific localization of PROM1 (green) in the developing human pancreas.

Confocal images of fetal pancreas sections (10.6 wpc). (D) MUC1, red. LAM (laminin), gray. DNA (DAPI), blue. (E) EZR, red. aPKC (atypical protein kinase C), gray. DNA (DAPI), blue. Scale bars, 25 μ m.

(F) Transgenic locus of the NEUROG3-TagRFPtEGFP reporter. TagRFPt, red fluorescent protein. NLS, nuclear localization signal.

Figure S4



Supplementary Figure S4. Identification and characterization of cell types by single-cell RNA sequencing, and single-cell RNA sequencing data validation, related to Figure 4.

(A) Clustering UMAP projections showing the entire dataset in all 4 samples. The samples are comprised of

endocrine, bipotent, ductal, and PSC-like populations.

(B) Expression of selected marker genes in each identified population. Log₂ fold change in count numbers relative to the average throughout the dataset is displayed.

(C) Allocation of hashtags to individual cells. Cells without hashtags or with two hashtags were excluded.

(D) Expression of selected marker genes in the endocrine subclusters. Log₂ fold change in count numbers relative to the average throughout the endocrine population is displayed.

(E) Enriched transcripts in 12 endocrine subclusters compared to the average throughout the endocrine population (log₂ fold change > 1, adjusted p-value < 0.0001). The 20 genes with the highest fold changes are shown.

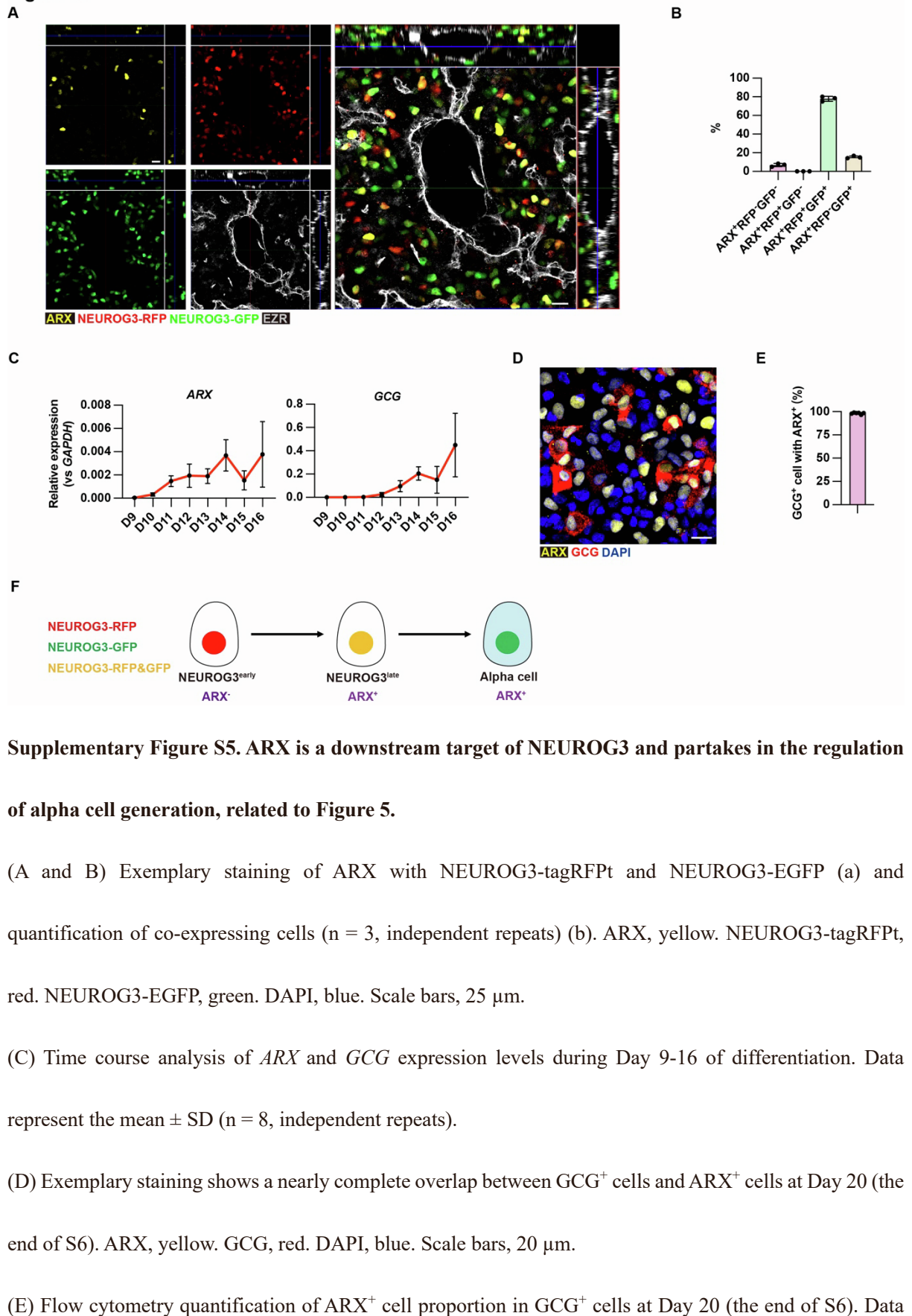
(F) Pseudo-time analysis of cells in the endocrine population.

(G) Validation of enriched top 3 transcripts identified in early-induced, late-induced, PROM1⁻ (non-polarized), and PROM1⁺ (polarized) EPs by quantitative real-time PCR. Data represent the mean ± SD (n = 7 for early and late-induced EPs; n = 5 for PROM1⁺ and PROM1⁻ EPs, independent repeats). *, p<0.05; **, p<0.01; ***, p<0.001 (two-tailed paired Student's t-test).

(H) Validation of *DLK1* expression by quantitative real-time PCR in NEG-siRNA and DLK1-siRNA cultures. Data represent the mean ± SD (n = 6 independent repeats).

(I) Flow cytometry quantification of the beta-to-alpha ratio in NEG-siRNA and DLK1-siRNA cultures on Day 17. Data represent the individual biological repeats (n = 6 independent repeats).

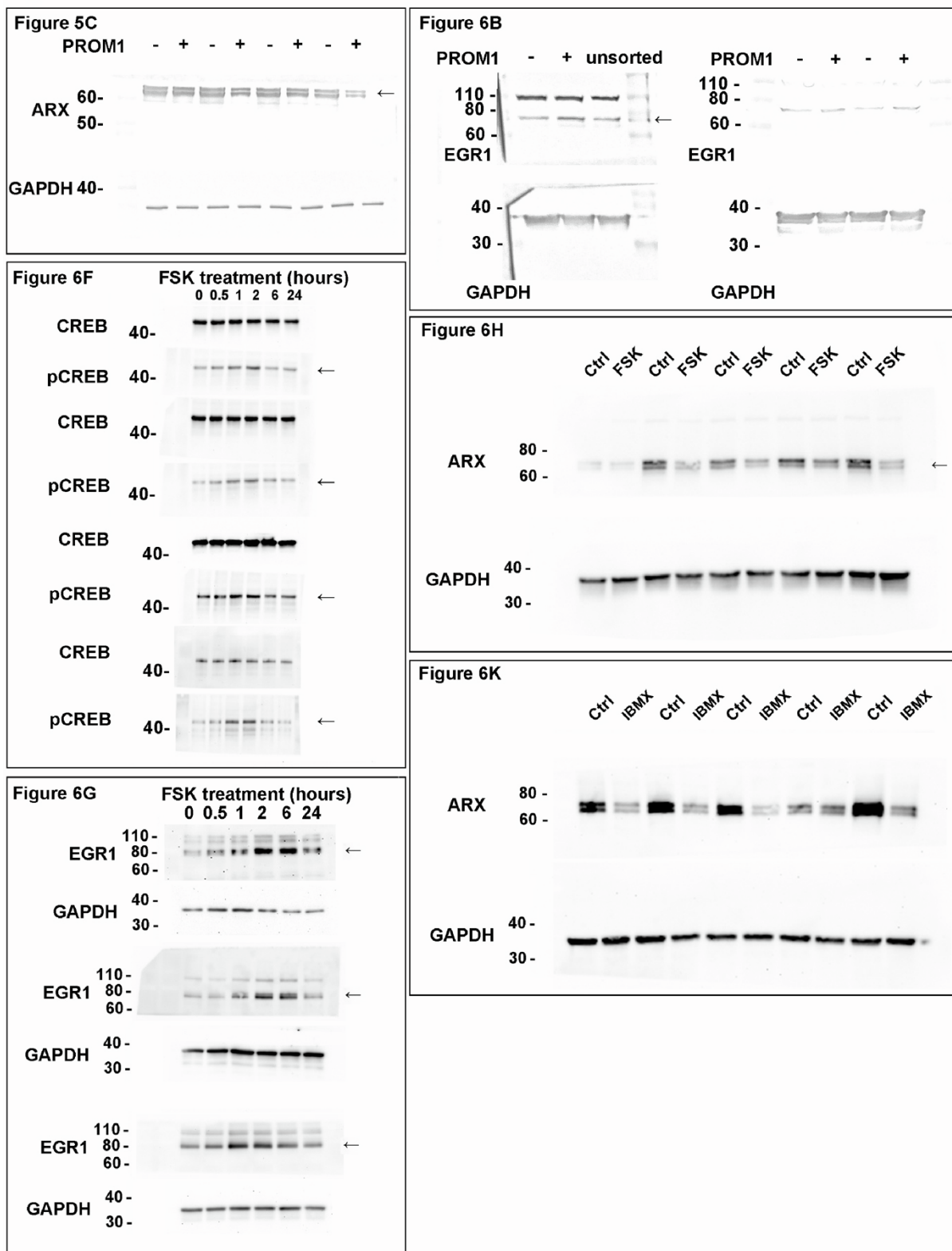
Figure S5



represent the mean \pm SD (n = 6, independent repeats).

(F) Model illustrating that ARX is absent in early NEUROG3⁺ EPs (TagRFpt⁺ and EGFP⁻) but starts expression in late NEUROG3⁺ EPs (TagRFpt⁺ and EGFP⁺). Later, ARX continues to be expressed in GCG⁺ alpha cells, which turn off TagRFpt but still express EGFP.

Figure S6



Supplementary Figure S6. Unprocessed Western blots, related to Figures 5 and 6.

Arrows point to the relevant band(s).

Supplementary Table S1. TaqMan probes and primers, related to STAR Methods.

TaqMan probe	identifier
<i>GADPH</i>	Hs02758991
<i>INS</i>	Hs02741908
<i>GCG</i>	Hs01031536
<i>DLK1</i>	Hs00171584
<i>GAST</i>	Hs01099852
<i>CHGA</i>	Hs00900370
<i>LDHA</i>	Hs01378790
<i>TFPI2</i>	Hs04334126
<i>SOX4</i>	Hs04987498
<i>LITDI</i>	Hs01102131
<i>PRSS2</i>	Hs00828418
<i>RGS5</i>	Hs01591223
<i>CRYBA2</i>	Hs00193234
<i>ARX</i>	Hs00292465
<i>EGR1</i>	Hs00152928
Primer	Sequence
<i>GADPH</i>	F: GGAGCGAGATCCCTCCAAAAT R: GGCTGTTGTCATACTTCTCATGG
<i>EGR1</i>	F: CCACGCCGAACACTGACATT

	R: GAGGGGTTAGCGAAGGCTG
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Supplementary Table S2. *In silico* transcription factor binding motif analysis for 2,000 base pairs upstream and downstream of the transcriptional start site of *ARX*, related to Figure 5.

Upstream	Downstream
<i>N-Myc</i>	<i>NURR</i>
<i>CLOCK:BMAL</i>	<i>SF1</i>
<i>USF</i>	<i>HNF4</i>
<i>Myc</i>	<i>FXR</i>
<i>USF1</i>	<i>NR1H2::RXRA</i>
<i>N-Myc</i>	<i>STAT5A</i>
<i>MYC::MAX</i>	<i>ETS2</i>
<i>alpha-CPI</i>	<i>NF-kappaB</i>
<i>NF-Y</i>	
<i>EGR1</i>	
<i>AHR</i>	
<i>ARNT</i>	
<i>HIF-1</i>	
<i>ETS2</i>	
<i>REST</i>	
<i>NRSF</i>	

<i>PURI</i>	
<i>MAFA</i>	